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MYCORRHIZA IN CERTAIN ERICACEAE NATIVE TO SOUTHERN AFRICA

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Abstract

The roots of selected species of *Erica* growing under natural conditions and in cultivation were examined to assess whether or not mycorrhizal associations were present. Infection was recorded in every case. The fungi necessary to bring about mycorrhizal formation are, therefore, of widespread occurrence in South African soils, and no specific host/fungus relationships appear to exist.

Uittreksel

MYCORRHIZA IN SEKERE SUID-AFRIKAANSE ERICACEAE. Die wortels van uitgesoekte spesies van ERIKA, wat onder normale omstandighede gegroei asook gekweek is, is ondersoek ten einde vas te stel of mycorrhizal verbindings teenwoordig is of nie. Besmetting is in elke geval gevind. Die fungus wat mycorrhizal formasies veroorsaak kom dus wyd versprei in Suid-Afrikaanse grond voor en geen spesifieke gasheer/fungus verhouding blyk te bestaan nie.

Introduction

Most of the families in the Ericales have been reported as including genera which are mycorrhizal, and the Ericaceae, in particular, are prone to this form of infection (Harley, 1969). The roots of this family are typically fine in structure, and, under natural conditions, many of them are surrounded by a loose weft of hyphae, which, in addition, may penetrate the cells of the cortex. Within these outer cells the hyphae proliferate to form compact 'clumps', but after a period of 'maturity' the hyphae are digested by the host cell. The balance between infection and digestion appears to depend on the vigour of the host, and hence the degree of infection will vary with growing season.

The fact that *Erica* spp. growing under natural conditions are infected in this manner has been interpreted as indicating that the presence of the fungus is essential for development of the host. (Rayner, 1925). The nature of the advantage derived by the host from the association is open to speculation, but, if the hypothesis is valid, then one would expect all species of heath to be infected. In addition, if a close relationship exists between host and fungus, it is possible that some degree of host specificity will exist among potential mycorrhizal fungi. If this were so, then the relatively local distribution of some heaths in South Africa might be explained, in part, by the absence of a suitable

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endophyte outside a specific geographical range. It is also possible that such specificity would raise problems for growers attempting to cultivate species for the consumer market.

It was decided to examine this question in relation to certain heaths in three ways:

(*a*) by examing root material of a number of *Ericas* both from natural locations and cultivated sites in South Africa;

(*b*) to grow a range of species from seed on soils known to have not recently supported an Ericaceous host, and to determine whether the roots of these plants become mycorrhizal;

(*c*) to isolate the endophytes from a number of South African heaths, and to test, under aseptic conditions, whether a particular isolate could produce normal mycorrhizal associations with test seedlings of other *Erica* spp.

Materials and Methods

Twelve species of *Erica* were selected, and root material was collected from specimens growing under natural conditions (see Table I). Roots were collected for examination by removing a core of soil, 1 in. × 6 in. (2,5 cm × 15,3 cm), from beside a selected plant. Three cores were obtained for each species. The material was soaked in water overnight to loosen the fine roots; these were then removed with forceps. Subsequently the roots from the three plants were bulked; one half of the sample was placed in fixative (formalin/acetic acid/ethanol (50%); 1:1:18), the other half was kept in distilled water at 5°C for a maximum of 24 hours prior to isolating the endophyte.

A similar procedure was followed for obtaining roots from equivalent specimens in cultivation in South Africa.

In the case of those species which were to be grown under experimental conditions, seed was obtained from the National Botanic Garden at Kirstenbosch. The seed was pre-germinated on moist, sterilized peat, and twelve seedlings were transferred to 6 in. (15,3 cm) pots containing a locally obtained acid soil (pH 4.2). The most vigorous seedlings were selected until only one plant remained in each pot. Six pots were planted for each species. The selected plants were maintained in the greenhouse with a mean air temperature of 16°C for twelve months.

The fine nature of the roots makes handling of individual specimens difficult, and accordingly small groups attached to a fine lateral root were embedded and sectioned (Johansen, 1940). The sections were stained in alcoholic chromotrope 2R (saturated soln.) for two hours. Clear differentiation of the endophyte within the cortical cells was obtained in this way.

The isolation of the endophytes was carried out using the technique of Bain (1937). A pure culture from each species of *Erica* was maintained on potato-dextrose agar for experimental purposes; only isolates from non-cultivated hosts were subsequently used.

To examined whether any host/fungus specificity existed, seed of the six species grown previously (see Table I) was surface sterilised with 10% calcium hypochlorite for 10 minutes: after washing in sterile distilled water, six seeds were transferred to the surface of agar slopes in test-tubes (2 cm diam.). The medium was 1% distilled water agar, as the use of this substrate avoids problems of nutrient toxicity, (Bain, 1937). This system was chosen to reduce the risk of extraneous infection (Brook, 1952), and in practice it was found that seedlings grown under these conditions for twelve weeks were comparable to soil-grown plants of the same age. The tubes, half-covered with black paper, were then placed in racks on a bench in a greenhouse (16°C). After one month the developing seedlings were inoculated by placing two inoculum discs (3 mm) of agar plus mycelium onto the surface of the water agar. The design used for carrying out the inoculations is shown in Table II. Ten tubes of each species of *Erica* were inoculated with each of the six available fungi. After two months the seedlings were removed and examined for mycorrhizal infection in the manner described previously.

RESULTS

The species examined for the presence of mycorrhizal infection are shown in Table 1.

TABLE 1

Origin of *Erica* spp. used in the current investigation.

Species	Origin	
	'Wild'	Cultivated
Erica blenna, *Salisb.*	Cape Province	Pretoria*
E. bauera. *Andre*	Cape Province	Pretoria
E. oatesii. *Rolfe*	Transvaal	Pretoria
E. inflata. *Thunb.*	Cape Province	Pot grown
E. ventricosa	Cape Province	Pot grown
E. daphniflora *Salisb.*	Cape Province	Pot grown
E. lateralis *Willd.*	Cape Province	Pot grown
E. mammosa *L.*	Cape Province	Pot grown
E. sessiliflora *L.*	Cape Province	Pot grown
E. campanularis *Salisb.*	Cape Province	Pretoria
E. cerinthoides *L.*	Cape Province	Pretoria
E. regia Bartl.	Cape Province	Cape Town†

* Gardens of the Botanical Research Institute.
† National Botanic Gardens, Kirstenbosch.
(Nomenclature follows Baker and Oliver, 1967).

A number of root samples from each species were examined, and in every case cortical cells showing the type of mycorrhizal infection normally associated with the Ericaceae were found. No attempt was made to assess the degree of infection displayed by a particular species, but two examples of the type of infection are shown in Plate: I. The frequency of infection suggests that the fungi capable of forming mycorrhizal associations with *Erica* spp. are present in a wide range of soils.

The fungi responsible for the endotrophic associations in the *Erica* spp. examined were basically similar in appearance, although some variation with respect to pigmentation was recorded. The colonies were composed of septate hyphae without clamp connections, and (when young) varied in colour from pale to dark grey. With age, the central region of the colony tended to become almost black, and, with some islates, semi-stromatic. The depth of colouration depended, in part, on the medium and was not a constant characteristic for

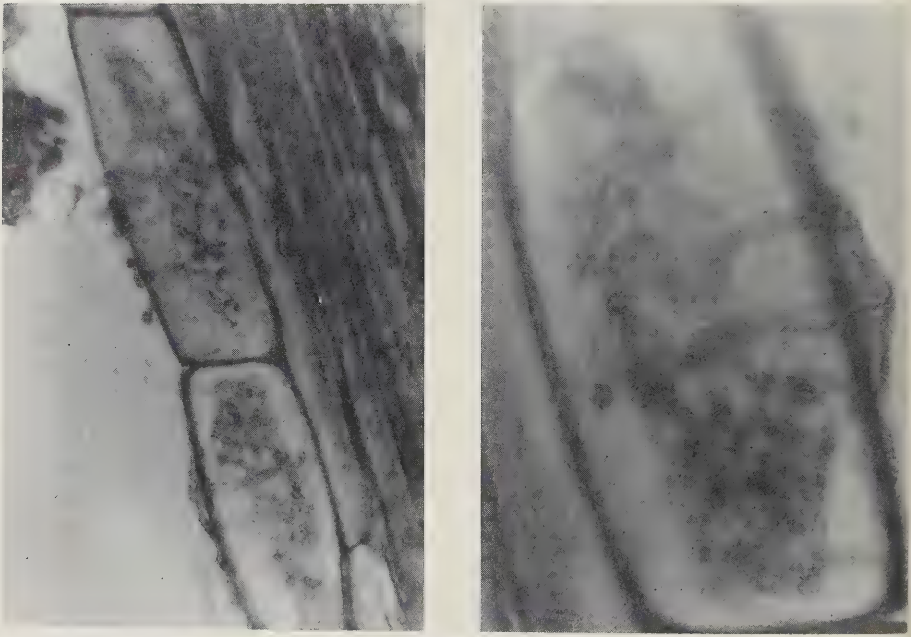

PLATE I

Roots of *Erica lateralis* showing typical mycorrhizal infection: (right x 1000) showing 'clump' of invading hyphae:
(left x 400) showing surface mycelium and penetration of the individual cells.

any particular isolate. The complete absence of fruiting structures meant that no exact systematic position could be established, although Booth (personal communication) tentatively identified an isolate from *Erica lateralis* as a species of *Hypoxylon* (Xylariaceae).

However, despite the apparent morphological similarity of the cultures examined, the variable degree of melanisation, for example, suggested that physiological differences might exist between isolates. Such variation might manifest itself also in relation to host specificity, and this idea was examined by testing the virulence of a particular fungus towards the *Erica* sp. from which it had been isolated, as well as towards species with which it had not had immediate contact. The results are shown in Table 2.

TABLE 2

Mean number of plants of *Erica* spp. showing mycorrhizal infection after being inoculated with the endophyte from another host.

	Test seedlings of:					
Endophyte isolated from:	Erica inflata	E. ventricosa	E. daphniflora	E. lateralis	E. mammosa	E. sessiliflora
Erica inflata	6	3	2	5	5	2
E. ventricosa	3	2	1	5	3	3
E. daphniflora	3	1	2	6	1	3
E. lateralis	5	2	2	4	2	1
E. mammosa	5	1	1	5	3	2
E. sessiliflora	1	3	6	5	2	3

In each case the assessment was based on the mean result of five tubes selected at random from each batch. Infection was recorded only when the roots of the seedlings showed typical mycorrhiza. It is clear from Table II that no precise relationship exists between the mycorrhizal fungi and their hosts.

DISCUSSION

The present investigation confirms that infection of *Erica* spp. in South Africa is a general and widespread occurrence, and that both wild and cultivated specimens become infected without difficulty. The apparently ubiquitous distribution of these associations suggests that the Ericaceae may, through the presence of the endophyte, be at a selective advantage in competing with other plant species. Thus, in typical fynbos vegetation, *Erica* spp. have been observed to be dominant in localised patches, and it is possible that selection is operating to form a more homogeneous community. However, such a view must remain mere speculation until it can be shown that the endophyte is materially important to the survival of the host.

It has been proposed by Morrision (1957) that the endophyte assists with nutrient absorption in the manner of the ectotrophic mycorrhizas of forest trees. However, this hypothesis lacks real experimental verification, as does the suggestion of Friesleben (1934), that the mycorrhizal fungi stimulate root growth by the removal of phytotoxic materials in the rooting medium. One last hypothesis which may merit further attention is that the mycorrhizal fungi, in conjunction with the host roots, produce fungitoxic metabolites (Robinson, 1972). These compounds might be effective, either in preventing the roots of *Erica* spp. being invaded by pathogens (Stalder & Schültz, 1957) or, by inhibiting the growth of other mycorrhizal fungi, in restricting the growth of certain plant species whose presence would be to the detriment of the Ericaceous shrubs (Handley, 1963).

However, whatever may be the true relationship between endophyte and host in *Erica* spp., it is clear that an understanding of the physiological basis of the association might go some way towards explaining the success of this genus under certain ecological conditions.

CONCLUSION

Certain species of *Erica* native of South Africa were found to have mycorrhizal infections irrespective of where they were grown. Thus, specimens in cultivation were just as heavily infected as individuals growing under natural conditions. It is clear, therefore, that the endophyte(s) enjoys widespread distribution, at least in acid soils, and that cultivation of *Erica* spp. either within the Republic or outside is unlikely to be hindered by the lack of a suitable mycorrhizal fungus. The precise identity of the fungal associates remains obscure, as does the real significance of the infection in relation to the host.

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